CLAIMS

What is claimed is:

1. A method for identifying oxidative modification of a protein, comprising:

generating a mass spectrum of all or a portion of a protein fraction derived from a biological sample, the protein fraction comprising at least one peptide that includes a nitrotyrosine residue, wherein determination of nitrotyrosine in said sample indicates the protein is oxidatively modified.

2. A method for identifying oxidative modification of a protein, comprising:

comparing (i) a first mass spectrum of all or a portion of a first protein fraction derived from a first biological sample, said first protein fraction comprising at least one peptide that includes a nitrotyrosine residue, to (ii) a second mass spectrum of all or a portion of a second protein fraction derived from a second biological sample, wherein determination of nitrotyrosine in said second protein fraction indicates that a protein therein is oxidatively modified.

3. A method for identifying oxidative modification of a protein, comprising:

contacting all or a portion of a protein fraction derived from a biological sample with at least one proteolytic agent under conditions and for a time sufficient to generate a plurality of peptide fragments derived from said protein fraction, the protein fraction comprising at least one peptide that includes a nitrotyrosine residue; and

generating a mass spectrum of one or more of said peptide fragments, wherein determination of nitrotyrosine in at least one of said peptide fragments indicates that a protein in the biological sample is oxidatively modified.

4. A method for determining protein tyrosine nitration in a subject, comprising:

isolating at least one protein comprising nitrotyrosine from a biological sample derived from a subject;

contacting the protein with at least one proteolytic agent under conditions and for a time sufficient to generate a plurality of peptide fragments derived from said protein; and

comparing a mass spectrum of one or more of said peptide fragments to a mass spectrum of a control sample containing nitrotyrosine, and therefrom determining protein nitration in the subject.

5. The method of any one of claims 1-4 wherein the mass spectrum is generated by matrix assisted laser desorption ionization mass spectrometry.

6. The method of claim 5 wherein determination of nitrotyrosine comprises detection in the mass spectrum of (a) a peptide comprising nitrotyrosine; (b) a peptide comprising nitrotyrosine that lacks one oxygen atom; and (c) a peptide comprising nitrotyrosine that lacks two oxygen atoms.

7. The method of any one of claims 1-4 wherein the mass spectrum is generated by matrix assisted laser desorption ionization time-of-flight mass spectrometry, and wherein determination of nitrotyrosine comprises detection in the mass spectrum of (a) a peptide comprising nitrotyrosine; (b) a peptide comprising nitrotyrosine that lacks one oxygen atom; and (c) a peptide comprising nitrotyrosine that lacks two oxygen atoms.

8. A method for identifying oxidative modification of a protein, comprising:

comparing (a) a first mass spectrum of a first portion of a protein fraction derived from a biological sample, wherein the protein fraction comprises at least one peptide that includes a nitrotyrosine residue, to (b) a second mass spectrum of a second portion of the

protein fraction derived from the biological sample, wherein the second mass spectrum is generated subsequent to exposure of said second portion to conditions sufficient to convert nitrotyrosine to aminotyrosine, wherein the second portion of the protein fraction comprises at least one peptide that includes an aminotyrosine residue derived from nitrotyrosine, and wherein determination of nitrotyrosine in said first portion and of aminotyrosine in said second portion indicates that at least one protein in the biological sample is oxidatively modified.

9. The method of claim 8 wherein prior to the step of comparing, the protein fraction is contacted with at least one proteolytic agent under conditions and for a time sufficient to generate a plurality of peptide fragments derived from said protein fraction.

10. The method of claim 8 wherein the peptide that includes an aminotyrosine residue derived from nitrotyrosine undergoes sidechain loss of aminotyrosine.

11. A method for identifying oxidative modification of a protein, comprising:

comparing (a) a first mass spectrum of a first portion of a protein fraction derived from a biological sample, wherein the protein fraction comprises at least one peptide that includes a nitrotyrosine residue, to (b) a second mass spectrum of a second portion of the protein fraction derived from the biological sample, wherein the second mass spectrum is generated subsequent to contacting said second portion with sodium dithionite under conditions and for a time sufficient to convert nitrotyrosine to aminotyrosine, wherein the second portion of the protein fraction comprises at least one peptide that includes an aminotyrosine residue derived from nitrotyrosine, and wherein determination of nitrotyrosine in said first portion and of amino tyrosine in said second portion indicates that at least one protein in the biological sample is oxidatively modified.

12. The method of claim 11 wherein prior to the step of comparing, the protein fraction is contacted with at least one proteolytic agent under conditions and for a time sufficient to generate a plurality of peptide fragments derived from said protein fraction.

13. The method of claim 11 wherein the peptide that includes an aminotyrosine residue derived from nitrotyrosine undergoes sidechain loss of aminotyrosine.

14. A method for detecting in a subject the presence of, or risk for having a disease associated with oxidative modification of a protein, comprising:

generating a mass spectrum of all or a portion of a protein fraction of a biological sample derived from a subject suspected of having or being at risk for having a disease associated with oxidative modification of a protein, the protein fraction comprising at least one peptide that includes a nitrotyrosine residue, wherein determination of nitrotyrosine in said sample indicates the protein is oxidatively modified, and therefrom detecting risk for or presence of a disease in the subject.

15. A method for detecting in a subject the presence of, or risk for having a disease associated with oxidative modification of a protein, comprising:

comparing (i) a first mass spectrum of all or a portion of a first protein fraction of a biological sample derived from a first subject suspected of having or being at risk for having a disease associated with oxidative modification of a protein, said first protein fraction comprising at least one peptide that includes a nitrotyrosine residue, to (ii) a second mass spectrum of all or a portion of a second protein fraction of a biological sample derived from a second subject known to be free of a presence or risk for having a disease associated with oxidative modification of a protein, said second protein fraction lacking nitrotyrosine, wherein determination of the presence of nitrotyrosine in said first protein fraction and the absence of nitrotyrosine in said second protein fraction indicates risk for having or presence of a disease in the first subject.

16. A method for identifying a protein that is oxidatively modified in a disease associated with oxidative modification of a protein, comprising:

comparing (i) a first mass spectrum of all or a portion of a first protein fraction of a biological sample derived from a first subject having or being at risk for having a disease associated with oxidative modification of a protein, said first protein fraction comprising at least one peptide that includes a nitrotyrosine residue, to (ii) a second mass spectrum of all or a portion of a second protein fraction of a biological sample derived from a second subject known to be free of a presence or risk for having a disease associated with oxidative modification of a protein, said second protein fraction lacking nitrotyrosine, wherein determination of the presence of nitrotyrosine in said first protein fraction and the absence of nitrotyrosine in said second protein fraction indicates risk for having or presence of a disease in the first subject; and

determining the protein from which said at least one peptide that includes a nitrotyrosine residue is derived, and therefrom identifying a protein that is oxidatively modified in the disease.

17. A method of identifying a suitable agent for treating a disease associated with oxidative modification of a protein, comprising:

comparing (i) a first mass spectrum of all or a portion of a first protein fraction of a biological sample derived from a subject having or being at risk for having a disease associated with oxidative modification of a protein, prior to contacting said sample with a candidate agent, said first protein fraction comprising at least one peptide that includes a nitrotyrosine residue, to (ii) a second mass spectrum of all or a portion of a second protein fraction of a biological sample derived from the subject subsequent to contacting said sample with the candidate agent, wherein determination of a decreased level of nitrotyrosine in said second mass spectrum relative to said first mass spectrum indicates the agent reduces oxidative protein modification.

18. A method of identifying a suitable agent for treating a disease associated with oxidative modification of a protein, comprising:

comparing at least one biological activity of a protein identified according to the method of claim 16 in the absence of a candidate agent to the biological activity of the protein in the presence of the candidate agent, wherein an alteration of said activity indicates suitability of the agent for treating a disease associated with oxidative protein modification.

19. A method for identifying oxidative modification of a proteome, comprising:

generating a mass spectrum of all or a portion of a protein fraction derived from a biological sample, the protein fraction comprising a plurality of proteins that each contain a nitrotyrosine residue, wherein determination of nitrotyrosine in said sample indicates the proteins are oxidatively modified.